Array Based Design of Multi-Wavelength Fluorescence System

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Abstract-**A method for exciting and collecting fluorescence from a surface using multiple excitations and emission wavelengths has been developed. Broadband excitation light is filtered to excite fluorophores in microbes. High efficiency collection reflectors allow detection of minimal amounts of microbial contamination. A working prototype is described.**

I. Introduction

In many industries today, there exists the need to detect contaminants on surfaces. Items, such as food preparation surfaces and hospital surgical equipment, should be free from microbial contaminants in order to assure the safety of personnel and consumers.

Detection of microbial contamination can be done using intrinsic fluorescent markers [1] without the need of any sample preparation, contact with any potentially contaminated surface, or lengthy processing time. Since the amount of fluorescence from a single microbe is very small, the optical design for such an instrument must be highly efficient and robust. All sources of optical noise must be considered and eliminated. This is a problem common to biomedical applications of fluorescence techniques.

II. Methodology

Optical materials become an issue as some of the fluorescent signals of interest are in the UV. Lens materials, such as UV fused silica and quartz, as well as a host of mirrors and specialty materials, all have intrinsic fluorescence signals well above tolerable levels. Highly polished metals do not suffer from the intrinsic noise limitation. Reflectors were designed and simulated using TracePro™ optical ray tracing software.

In order to minimize the complexity of the overall mechanical design and to ensure continuity between measurements, it was decided to illuminate the surface of interest with all three excitation wavelengths simultaneously. Six or eight emission wavelengths are collected. Bandpass interference filters are used for both excitation and emission. The interference filters have a band pass width of 10nm FWHM with 40% attenuation at the peak and an out-of-band pass attenuation of 10^{-6}. Multiple filters increase the selectivity, but also attenuate the signal.

These filters require the incident light to be perpendicular to the filter surface. If the angle of incidence is greater than 10°, the band-pass shape deteriorates and the peak pass is shifted. In order for the simultaneous collection scheme to be effective, all of the light must be collimated and perpendicular to the surface of the filter.

A. Excitation Optics

Broad band illumination from a xenon flash lamp is collimated using a fused silica lens and passed to the three excitation filters. Since each wavelength should illuminate the same spot on the sample surface, collimated light from each filter must be focused onto the sample (Figure 1). Polished metal mirrors are used to overlay and focus the three collimated excitation beams.

A two surface focusing element is used to provide the focusing where each surface has a single curvature. After the excitation filter, each beam of excitation light hits a polished and chromed aluminum mirror which has a curvature in the X-direction and is flat in the Y- direction and is mounted at 45° to the incident light. The light is passed to another mirror at the appropriate angle so to point the light on the desired spot on the sample, with a curvature in the Y-direction and flat in the X-direction.

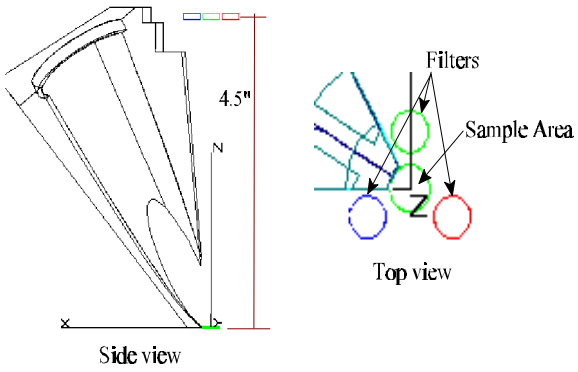

Fig. 1. Excitation Filter Geometry

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Abstract

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B. Emission Collection

The sample emits fluorescence spherically and this fluorescence must be collimated for the interference filters and then focused on the PMT active surface. A paraboliod reflector allows ~90% of the rays emitted from the focal point to be collimated after reflection off the parabolic surface. By making the axial length of the reflector 100 times larger than the focal length, the majority of the fluorescence photons "appear" to have come from the focal point. The sample is placed in the focal plane.

Six or eight separate paraboloids were arranged such that each shared the same focal point at the sample surface. These were arranged to collect light from as much of the 180 steradian emission as possible. At the end of each reflector are the bandpass filters, followed by a focusing lens and photodetector (Figure 2).

III. Results and Discussion

Using TracePro™, individual optical elements were built from the optical models. With a sample area of ~ 0.585cm² overall collection, the simulated collection efficiency of the emission system is 89%. Using a differential dilution test and back-calculating through the transfer function of the associated instrumentation, the collection efficiency of the optical pieces was found to be ~85%.

The designed reflector was built out of brass and chrome plated to give the necessary reflectivity. Figure 3 shows the fluorescence collected from cells on a reflective surface (~0.6 cm²). Note that the behavior is linear as a function of concentration of cells and that as few as ~10 cells can be detected with this design.

V. Conclusions

The excitation and collection of very small UV fluorescence signals from a surface is a challenging problem. Excitation and emission optical elements must be reflective and made from materials which do not fluoresce. Collection and collimation of all fluorescence photons is achieved using a set of common focal point paraboloid reflective elements. High collection efficiency and low optical noise input allows the detection of small numbers of microbes on a surface.

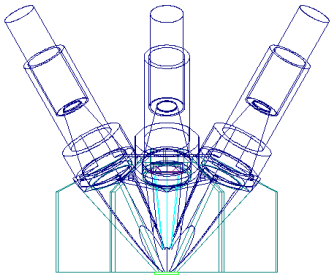

Fig. 2. Emission system with parabolic reflectors

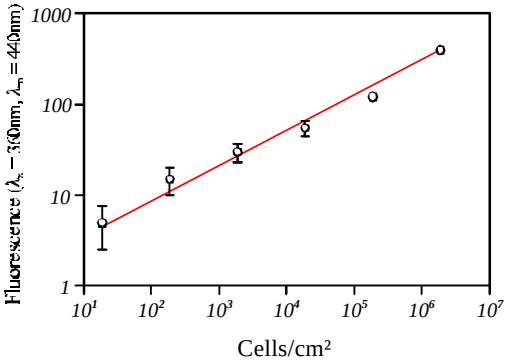

Fig. 3. Cells on a surface

Acknowledgments

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References

[1] Estes, C., Duncan, A., Wade, B., and Powers, L., Real-Time Multiwavelength Microbe Detection Instrument, Abstract for Poster Presentation at the EMBS/BMES Joint Conference, Atlanta, GA, Oct. 13-16, 1999.